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EXTRACTION OF THE GROWTH REGULATOR UNICONAZOLE (XE-1019) FROM SILVER MAPLE WOOD

Report No 90-40-K

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ABSTRACT

Silver maple saplings were trunk-injected with the growth regulator uniconazole (^{14}C-labelled). Wood taken from just above the injection point of saplings harvested after 0, 134 or 500 days contained about 95% uniconazole, with little label in metabolites. A short incubation with water extracted more uniconazole from day 0 wood than day 134 or 500. The proportion of uniconazole extracted by various solvents remained constant, not affected by the initial uniconazole concentration in the wood or the wood harvest date.

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EXECUTIVE SUMMARY

EXTRACTION OF THE GROWTH REGULATOR UNICONAZOLE (XE-1019) FROM SILVER MAPLE WOOD

Growth regulators, such as paclobutrazol, flurprimidol and uniconazole, have been reported to inhibit the growth of many plant species, including trees. Application of such products to trees under or beside distribution lines may result in a reduction of the frequency of trimming of such trees. A popular method of application of these products is trunk injection. This method has advantages over other methods (e.g. bark banding, basal drench, soil injection, foliar spray) in that the amount applied is precise, chemical contact with non-target plants is prevented and environmental residues are reduced. After trunk injection the growth regulators paclobutrazol, flurprimidol and uniconazole remain around the injection site, with little translocated up the stem and into the foliage or down into the roots. In studies conducted for a month, about 10% of each applied growth regulator was found in the stem and new growth (shoots including foliage) away from the injection point. In an independent study conducted for 500 days with uniconazole, most of the applied growth regulator was found within 20 cm of the injection point. The foliage produced in the first growing season after injection contained 7.2 ± 1.5% of the applied uniconazole, with 2.3 ± 0.6% in the foliage produced in the second growing season.

The apparent lack of movement of such growth regulators from the injection site area might be attributed to their low solubility in water (35, 130 and 15 ppm for paclobutrazol, flurprimidol and uniconazole, respectively) although the volume of water passing up the plant in an entire growing season will be extremely large. Alternatively, the growth regulators could be metabolized (with time) to products which are less soluble in water and/or bind to components of wood more tightly. In short-term studies the rate of metabolism of these growth regulators around the injection site

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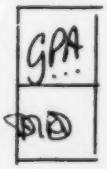
appears to be low, although uniconazole has been shown to be metabolized in new growth (shoots plus foliage) and foliage alone over the longer-term (data from independent studies). Over four months approximately 40% of uniconazole was metabolized.

In the present study the foliage of silver maple saplings harvested in the third growing season after trunk injection of ^{14}C-uniconazole contained 1.2 ± 0.2% of the applied material (compared with 7.2 ± 1.5% and 2.3 ± 0.6% in the first and second growing season, respectively). Sprout growth was inhibited in the third season after injection. The proportion of uniconazole and metabolites in wood from close to the injection site of saplings harvested 0, 134 and 500 days after trunk injection was determined. Between 95 and 97% of the ^{14}C-activity found in this wood chromatographed with uniconazole, indicating little or no metabolism of the growth regulator had occurred over 500 days. In foliage from saplings harvested at 133 and 497 days slightly less of the ^{14}C-activity chromatographed with uniconazole. The proportion of ^{14}C-activity that was extracted from this wood by various solvents was also determined. Water extracted about 20% of the ^{14}C-activity from day 0 wood in a 30 second incubation experiment, but only about 10% of the activity from day 134 or day 500 wood. With longer incubation times there was no difference. The time of harvest also had no effect on the proportion of ^{14}C-activity extracted by various organic solvents. Nonpolar solvents extracted a larger proportion of the activity in day 0 wood than from day 134 or day 500 wood. The proportion of ^{14}C-activity that was extracted by the various solvents did not change either as a function of the initial amount of ^{14}C-activity in the wood, or the time of harvest of the wood. This would suggest that as the concentration of uniconazole in the wood declines as a result of translocation, the percentage of uniconazole available for translocation remains constant.



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To Dr. D. Mills
Director of Research

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1.0 INTRODUCTION

Growth regulators, such as paclobutrazol (PP-333) (2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-ol), flurprimidol (EL-500) (α-(1-methylethyl)-α-(4-(trifluromethoxy)phenyl)-5-pyrimidine methanol) and uniconazole (XE-1019){(E)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-pentan-3-ol} have been reported to inhibit tree growth in many studies (see for example Miller, 1982; Hare, 1984; Aron et al., 1985; Arron, 1985, 1986; Sterrett, 1985, 1988; Greene, 1986; Marini, 1986; Sterrett and Tworkoski, 1987; Gilliam et al., 1988; Wood, 1988; Steffens, 1988). Application of such products to trees under or beside distribution lines may result in a reduction in the frequency of trimming of such trees. One of the most popular methods of application of these products is by trunk injection, and this method has advantages over other methods (such as bark banding, basal drench, soil injection, foliar spray) in that the amount applied is precise, chemical contact with non-target plants is prevented and environmental residues are reduced (Sterrett, 1988).

After growth regulators have been applied to trees by trunk injection, they remain around the injection site with little translocated up the stem and into the foliage. Sterrett and co-workers (Sterrett, 1985, 1988; Sterrett and Tworkoski, 1987) who performed short-term studies with ^{14}C-labelled paclobutrazol, flurprimidol and uniconazole applied to apple seedlings, reported that less than 10% of the applied label was found in the stem and new growth (shoots including foliage) away from the injection point, and no downward movement into the roots was detected up to about 1 month after application. Most of the ^{14}C-activity was found in the stem within 20 cm of the injection site. A more recent long-term study (Arron, 1989b) revealed that up to 500 days after injection of ^{14}C-uniconazole into silver maple (Acer saccharinum L.)

saplings most ^{14}C-activity was detected in a 45 cm section of stem, with the injection point in the middle, and very little was found either higher up the stem, in the foliage, or in the roots. Of the ^{14}C-activity found in parts of the saplings other than the 45 cm stem section, most was detected in the foliage, where 7.2 ± 1.5% and 2.3 ± 0.6% of the total ^{14}C-activity applied was located in 1987 and 1988 respectively. The earlier work of Sterrett (1988) with labelled uniconazole has been extended and by 4 months after injection he found that 16% of the ^{14}C-activity recovered was in new growth (Sterrett, 1989). In such time-course experiments the recovery (or retrieval) of ^{14}C-activity becomes less and less efficient (see Sterrett, 1985; Sterrett and Tworkoski, 1987; Arron, 1989b), and this would mean that the 16% of recovered ^{14}C-activity would be equivalent to less than 10% of the total ^{14}C-activity applied.

From the studies described above it is apparent that following trunk injection the growth regulators paclobutrazol, flurprimidol and uniconazole all remain around the injection site with little translocated to other parts of the plant. The apparent lack of movement of these growth regulators may be attributed to their low solubility in water (35, 130, and 15 ppm respectively, see Anon, 1983, 1984, 1986), however the amount of water passing up the stem in the transpiration stream would be extremely large. The growth regulators might be located in areas of discoloured wood which have been compartmentalized off. Both Wright and Moran (1989) and Houde (1988) have observed discoloured areas in trees trunk-injected with growth regulators. Growth regulators in these areas may be less mobile because water flow through them is reduced. More work needs to be done on tree wounding and the long-term effect of discoloured wood. With time the growth regulators might become more and more tightly bound to components of the wood. Such a situation has been reported for herbicides applied to soils, where McCall and Agin (1985) found that the amount of picloram (4-amino-3,5,6-trichloropicolinic acid) readily released from soil decreased significantly with the length of time the herbicide had been incubated with the soil. However soils are more complex media than wood. Alternatively growth regulators could be metabolized (with time) to products which are less soluble in water and/or bind to components of wood more tightly. Sterrett (1988) reported that uniconazole was stable in new growth and in the phloem and xylem of the stem for the first 28 days after injection. In longer-term studies metabolites of uniconazole have been reported in new growth (Sterrett, 1989) and in foliage (Arron, 1989b). In the latter study it was concluded that the primary location of uniconazole metabolism was probably the foliage rather than the stem (around the injection site) because the proportion of ^{14}C-activity found in metabolites in the foliage did not increase from the first growing season post-injection to the second season. Both paclobutrazol and flurprimidol have been shown to be inhibitory to the growth of trees for two, three and four years post-injection, and this would suggest that metabolism of these growth regulators into non-

inhibitory products did not occur on a large scale (Wood, 1988; Gilliam et al, 1988; Arron, 1989a).

Information on the metabolism of uniconazole in wood around the injection point and in foliage would be useful when determining the dose to apply to a tree to inhibit growth over the longer term. The present study was designed to determine the proportion of uniconazole and metabolites in wood found close to the injection site in silver maple saplings harvested 0, 134 and 500 days after trunk injection with ^{14}C-uniconazole. The availability of uniconazole in this wood was also determined by performing extractions with water and other solvents. Data are also presented on the effects of uniconazole on the growth of silver maple saplings in the third growing season post-injection and the translocation of uniconazole into the foliage of these saplings. Data on growth and translocation in the first and second growing seasons have been presented already (Arron, 1989b).

2.0 MATERIALS AND METHODS

2.1 Plant Material, Trunk Injection with ^{14}C-uniconazole, Collection of Foliage and Stem Samples

Silver maple (Acer saccharinum L.) saplings, growing at Wesleyville GS, were used in both the foliage uptake experiment and the wood extraction experiment. The four-year-old saplings were planted in a grid (4 x 4 m spacing) with saplings in one half of the grid used in the foliage uptake experiment and those in the other half used in the extraction experiment. Within the two halves of the grid, saplings were selected at random and then all trimmed and trunk-injected with either ^{14}C-uniconazole or isopropyl alcohol (control saplings) (see Arron, 1989b). In the foliage uptake experiment five saplings were injected with ^{14}C-uniconazole and five with isopropyl alcohol. Leaves (30 picked at random) were collected from each of these ten saplings immediately post-injection (day 0) and then 7, 17, 25, 35, 45, 65, 85, 101, 122, 133, 375, 416, 444, 472, 497, 736, 757, 802, 836 and 861 days later. At the end of each growing season (days 133, 497 and 861) all foliage left on the saplings was removed. On the same days the length of all sprouts formed in that growing season was determined. Sprouts were measured to the nearest 0.5 cm, and those with a length of 1 cm or less were recorded as being 1 cm in length. Foliage samples were frozen and stored (-16°C) prior to determination of ^{14}C content.

In the wood extraction experiment twelve saplings were injected with ^{14}C-uniconazole and six with isopropyl alcohol. Translocation of ^{14}C-uniconazole within these saplings was determined in a previous study (see harvest experiment - Arron, 1989b). Three saplings were harvested (foliage, stem and roots) at either 0, 134 or 500 days after injection. Sections of sapling stem, extending from 7.5 cm to 22.5 cm above the injection point, were frozen (-16°C) and stored until used in the extraction experiment (see

below).

2.2 Determination of ^{14}C Content of Foliage and Stem Samples

Foliage, either the 30 leaf samples collected during the growing seasons or the foliage that remained at the end of the growing season, was thawed and homogenized with distilled water using a Polytron or Vertis. The homogenate was frozen overnight (-16°C) and freeze dried. About 0.5 g (DW) of homogenate was made into a pellet and oxidized (Packard Model 306 oxidizer) (Peterson, 1969). The amount of $^{14}CO_2$ evolved after oxidation was determined by liquid scintillation spectrometry (Packard Tri-Carb 300) using Permafluor V. Freeze dried foliage homogenates were retained for metabolite estimation (see below). The 15 cm long stem samples were chopped into small pieces, freeze dried, ground up (coffee grinder) and passed through a Wiley Mill (#40 screen) to produce a fine sawdust. Approximately 0.4 g (DW) was mixed with 0.1 g cellulose powder (to ensure an even burn in the oxidation step), made into a pellet and oxidized.

2.3 Separation of ^{14}C-Uniconazole and Metabolites

In the determination of the proportion of ^{14}C-activity present in uniconazole and metabolites, samples of freeze dried sawdust (made from stem samples) and foliage homogenates were extracted with methanol (2x with 50 mL 80% methanol-see Sterrett, 1988). With sawdust 0.2 g (DW) was extracted. Distilled water (25 mL) was added and the extract evaporated to an aqueous solution. The pH was adjusted to 7.0 by adding distilled water (pH 12.0 adjusted with KOH), and the extract partitioned (3x) with 50 mL of methylene chloride. The pooled methylene chloride fractions were evaporated almost to dryness and the residue taken up in acetone. The extracts were applied to TLC plates (Redi-Plate Silica Gel GF 250 µm; Fisher Scientific) and the chromatograms developed for 15 cm in chloroform: ethyl acetate: acetic acid (3:6:1 by volume) (Sterrett, 1988). Developed chromatograms were divided into 10 segments, the gel scraped off and the ^{14}C content quantified by liquid scintillation spectrometry using Picofluor 40. The method was basically the same with foliage except that 10 g of freeze-dried foliage homogenate was extracted in 100 mL of methanol. After evaporation of the pooled methylene chloride fractions the residue was taken up in about 8 mL of methylene chloride and 1 or 2 mL loaded onto a 900 mg Florisil SPE column (Waters Scientific) (Booth et al, 1989). The column had been first conditioned with 6 mL methylene chloride. The sample was rinsed with 6 mL methylene chloride before eluting with 25 mL anhydrous ethyl ether which was collected and evaporated to dryness. The residue was taken up in about 2 mL of acetone and applied to TLC plates as described above. Most of the chlorophyll remained on the Florisil column and this meant little was applied to the TLC plate.

2.4 Solvent Extraction Studies

Two different experiments were performed with the sawdust prepared from the sapling stem samples. In a time-course experiment 0.1 g of sawdust was mixed with 50 mL of distilled water (adjusted to pH 7.0) and shaken for 0.5, 0.75, 1.0, 1.5, 2, 5, 10, 30, 60 or 120 minutes. For each sapling triplicate sawdust samples were extracted. A preliminary experiment conducted with 50 mL of 0.2 M phosphate buffer (Dawson et al., 1969) in the range pH 5.8 to 7.8 indicated that the amount of ^{14}C-activity released was pH independent. Using volumes of water greater than 50 mL did not result in the release of more ^{14}C-activity, but with less than 50 mL less ^{14}C-activity was released, presumably because the water was saturated.

3.0 RESULTS AND DISCUSSION

3.1 Effect of Trunk Injection of ^{14}C-Uniconazole on the Growth of Silver maple saplings in the 1987, 1988 and 1989 Growing Seasons

The effects of trunk injection of uniconazole on the number of sprouts found on the silver maple saplings in the 1989 growing season are shown in Table 1. Data from the 1987 and 1988 growing seasons are shown for comparison (Arron, 1989b). The saplings had been trimmed and trunk-injected with ^{14}C-uniconazole in May 1987 and the translocation of ^{14}C-activity into the foliage determined in subsequent growing seasons (see below). Uniconazole application had no effect on the number of sprouts found per sapling compared to the control (Table 1). Separating the sprouts into those found above the injection point or below also revealed no effect of the growth regulator. From 1987 to 1989 there was an apparent increase in the number of sprouts per sapling in both treated and control saplings, and this reflects an increase in overall canopy size.

The effects of uniconazole on the length of sprouts formed above the injection point in the 1989 growing season are shown in Table 2. Again data from 1987 and 1988 are included for comparison. In both 1988 and 1989 an inhibition of sprout growth was observed (Mann-Whitney non-parametric test). In both years the median sprout length was consistently 1.0 cm for saplings treated with uniconazole. In short-term studies (one growing season or less) inhibition of growth by uniconazole has been reported before (Sterrett, 1988; Liyembani and Taylor, 1989).

3.2 ^{14}C-Activity in the Foliage of Silver Maple Saplings following Trunk Injection with ^{14}C-Uniconazole

Changes in the ^{14}C-activity in the foliage of silver maple saplings following trunk injection with ^{14}C-uniconazole are shown in Table 3. The ^{14}C-activity is expressed as specific activity (Bq/g dry

weight) and similar increases were obtained when ^{14}C-activity was expressed on a per leaf basis, as there was little change in leaf dry weight over the course of the experiment. As discussed previously (Arron, 1989b) there was an increase in the concentration of ^{14}C-activity in the foliage up to 134 days after injection, and in the second growing season a similar increase was observed, although the maximum concentration reached was only one third that reached in the first season. In the third growing season the result was the same with the highest concentration reached being approximately one sixth that of the first year. From these data it appeared that less ^{14}C-activity was translocated into the foliage each year and this was confirmed when the total ^{14}C-activity was calculated for all the foliage removed during 1989 (30 leaf samples removed during growing season plus all remaining leaves stripped off in the fall) (see Table 4). In the 3 years just over 10% of the ^{14}C-activity injected into the saplings was translocated into the foliage with most appearing in the foliage in the first year. The total number of leaves collected and their dry weight did not change significantly over the three years. All previous studies, where the translocation of paclobutrazol, flurprimidol or uniconazole was followed after trunk injection were terminated after 4 months or less. Sterrett (1989) reported that 16% of the ^{14}C-activity recovered 4 months after injection of apple (Malus domestica Borkh) saplings with ^{14}C-uniconazole was in new growth (shoots including foliage). Recovery of ^{14}C-activity declines the longer the experiment (Sterrett, 1985; Sterrett and Tworkoski, 1987; Arron, 1989b) and therefore the 16% of ^{14}C-activity quoted by Sterrett (1989) would be about 10% when expressed as a function of the total ^{14}C-activity injected into the saplings. In the first and second year post-injection the concentration of ^{14}C-activity in the young sprout tissue was lower than that found in the foliage (Arron, 1989b) and the situation was probably the same in the third year. The concentration of uniconazole in the young sprout tissue was high enough to result in an inhibition of growth in the third year (Table 2).

3.3 Metabolism of ^{14}C-Uniconazole in Wood collected 0, 134 and 500 days after Trunk Injection of Silver Maple Saplings with ^{14}C-Uniconazole and in Foliage Collected after 133 and 497 days.

In the translocation results described above the ^{14}C-activity found in the foliage represent the total activity in uniconazole and/or metabolites formed from uniconazole. To determine the proportion of activity in uniconazole and metabolites, extracts were prepared form wood collected on day 0 (immediately post-injection), 134 and 500, and from foliage collected on day 133 and 497. In the extracts of wood, between 95 and 97% of the ^{14}C-activity detected was uniconazole (R_f = 0.7 to 0.9) (see Table 5). Sterrett (1988) reported that in an experiment terminated at 28 days, between 96 and 99% of the ^{14}C-activity detected in extracts of phloem and xylem

TABLE 1

EFFECT OF TRUNK INJECTION OF ^{14}C-UNICONAZOLE ON THE MEAN NUMBER OF SPROUTS FORMED ON SILVER MAPLE SAPLINGS IN THE FOLIAGE UPTAKE EXPERIMENT IN THE 1987, 1988 AND 1989 GROWING SEASONS*

Treatment	Growing Season	Number of Sprouts per Sapling		
		All Sprouts	Above Injection Site	Below Injection Site
		----------	---------(mean ± s.d.)*----------	----------
Control	1987	74 ± 24	69 ± 24	5 ± 6
Uniconazole	1987	89 ± 37	75 ± 25	14 ± 13
Control	1988	150 ± 91	147 ± 91	3 ± 3
Uniconazole	1988	130 ± 41	123 ± 39	7 ± 5
Control	1989	188 ± 87	186 ± 89	3 ± 4
Uniconazole	1989	173 ± 72	165 ± 67	9 ± 8

*The mean was calculated from five saplings in the control treatment and four saplings in the uniconazole treatment. [One of the five saplings injected with uniconazole did not receive the full dose of the growth regulator, ie. there were problems with the injection procedure (see Arron, 1989b)].

*Data for 1987 and 1988 taken from Arron (1989b).

TABLE 2

EFFECT OF TRUNK INJECTION OF ^{14}C-UNICONAZOLE ON THE LENGTH OF SPROUTS FORMED ABOVE THE INJECTION SITE ON SILVER MAPLE SAPLINGS IN THE FOLIAGE UPTAKE EXPERIMENT IN THE 1987 AND 1989 GROWING SEASONS - MEDIAN VALUES*

Treatment	Sapling	Median Sprout Length (cm)**		
		1987	1988	1989
Control	K1	2.00	2.00	2.50
Control	J1	1.50	1.75	2.00
Control	J7	2.75	1.00	1.00
Control	H2	2.75	2.00	1.50
Control	I5	2.00	1.50	1.50
Uniconazole	K4	2.50	1.00	1.00
Uniconazole	J3	1.50	1.00	1.00
Uniconazole	I6	2.00	1.00	1.00
Uniconazole	I3	1.00	1.00	1.00

*Data for 1987 and 1988 taken from Arron (1989b).
**Sprouts were measured as described in Materials and Methods

TABLE 3

INCREASE IN ^{14}C-ACTIVITY IN FOLIAGE COLLECTED IN 1987, 1988 AND 1989 FROM SILVER MAPLE SAPLINGS TRUNK-INJECTED WITH ^{14}C-UNICONAZOLE (FOLIAGE UPTAKE EXPERIMENT)

GROWING SEASON					
1987[a]		1988[a]		1989	
DAYS AFTER INJECTION	^{14}C-ACTIVITY*	DAYS AFTER INJECTION*	^{14}C-ACTIVITY*	DAYS AFTER INJECTION*	^{14}C-ACTIVITY*
0	-0.1 ± 0.2	375(0)	1.7 ± 1.4	736(0)	1.1 ± 0.7
7	1.5 ± 1.6				
17	4.2 ± 3.1				
25	10.2 ± 6.0			757(21)	2.5 ± 1.6
35	26.4 ± 13.6				
45	27.3 ± 19.9	416(41)	19.3 ± 5.6		
65	49.2 ± 30.9	444(69)	25.3 ± 12.8	802(66)	7.5 ± 4.5
85	59.7 ± 22.8				
101	61.4 ± 40.1	472(97)	26.9 ± 14.6	836(100)	13.1 ± 7.8
122	81.8 ± 25.8	497(122)	20.3 ± 11.8	861(125)	10.9 ± 2.9
133	81.7 ± 30.8				

Bq/g Dry Weight; values given are mean±s.d (n=4).

*Value in brackets represents days over the particular growing season, with day 0 being in the third or fourth week of May. Day 0 in 1987, when saplings were injected was May 25th. Foliage was present on the saplings at that time (otherwise no foliage samples could be collected).

[a]Data for 1987 and 1988 from Arron (1989b).

TABLE 4

PROPORTION OF ^{14}C-ACTIVITY FOUND IN THE FOLIAGE OF SILVER MAPLE SAPLINGS TRUNK-INJECTED WITH ^{14}C-UNICONAZOLE (FOLIAGE UPTAKE EXPERIMENT)**

Year	Total Number of Leaves Collected per Sapling (mean ± s.d.)*	Total Dry Weight of Leaves Collected per Sapling (g) (mean ± s.d.)*	Total ^{14}C-Activity in Leaves, per Sapling (Bq) (mean ± s.d.)*	^{14}C-Activity in Leaves Collected as Function of Amount Injected (%) (mean ± s.d.)*
1987	800 ± 175	173 ± 35	9193 ± 1972	7.2 ± 1.5
1988	980 ± 344	156 ± 47	2972 ± 734	2.3 ± 0.6
1989	1044 ± 357	164 ± 45	1532 ± 283	1.2 ± 0.2

*Mean value calculated from four saplings.

**Data for 1987 and 1988 taken from Arron (1989b).

respectively, chromatographed with uniconazole. Similar results had been reported earlier for both paclobutrazol and flurprimidol (Sterrett, 1985; Sterrett and Tworkoski, 1987). The results from the present study indicate that uniconazole present in wood found just above the injection point in silver maple saplings was not metabolized for up to 500 days after injection of the growth regulator. In the extracts from foliage collected on day 133 and 497 between 83 and 88% of the ^{14}C-activity chromatographed with uniconazole (see Table 6). These extracts were prepared differently from those reported earlier (Arron, 1989b), although samples of the same freeze-dried homogenates were extracted. In the earlier report between 53 and 71% of the ^{14}C-activity chromatographed with uniconazole. This apparent discrepancy can be explained by the chlorophyll contamination of the earlier extracts which made running the TLC plates difficult. In the present study the extracts were run through Florisil columns which removed almost all the chlorophyll.

3.4 Extraction by Various Solvents of ^{14}C-Activity from Wood of Silver Maple Saplings harvested 0, 134 and 500 days after Trunk Injection with ^{14}C-Uniconazole

Wood from saplings harvested 0, 134 and 500 days after injection of the sapling with ^{14}C-uniconazole was extracted with water in a time-course experiment and with a variety of other solvents in a second experiment. In the time-course experiment there was an increase in the proportion of ^{14}C-activity extracted from the wood as a function of the time of incubation (see Figure 1). After a 30 second incubation with water, about 20% of the ^{14}C-activity present in the wood from saplings harvested on day 0 was extracted, while about 10% of the ^{14}C-activity was extracted from wood of saplings harvested on day 134 and 500 (see inset in Figure 1). The differences between the releases of ^{14}C-activity from wood of day 0 saplings and day 134 and 500 saplings was most obvious with short incubation times, and by 120 minutes there appeared to be no differences. All three extraction curves were flat at the longer incubation times, and experiments with even longer incubation times (24 hours) revealed no significant increase in the ^{14}C-activity extracted (data not shown). This would suggest that equilibrium was reached between ^{14}C-activity in solution and ^{14}C-activity bound to wood components in about 2 hours. Time of harvest post-injection had no effect on the proportion of ^{14}C-activity that was not extracted by water, with about 40% remaining unavailable for all three harvest times. With short incubation times ^{14}C-activity was extracted more readily from wood of saplings harvested immediately after injection. This would suggest that following injection of uniconazole into the saplings there was a time dependent reduction in the amount of growth regulator that was readily soluble. Interpretation of these results is difficult in that within the sapling the growth regulator may be exposed to a passing stream of water, a different situation from the simple

TABLE 5

DISTRIBUTION OF ^{14}C-ACTIVITY IN EXTRACTS OF WOOD FROM SILVER MAPLE SAPLINGS TRUNK-INJECTED WITH ^{14}C-UNICONAZOLE

Fraction on Chromatography Plate Counted (Rf values)	Days After Injection					
	0		134		500	
	Extract	Uniconazole Reference	Extract	Uniconazole Reference	Extract	Uniconazole Reference
	%^{14}C-Activity detected on Plate*					
0.0 - 0.1	1.0	0.1	0.8	0.1	0.5	0.1
0.1 - 0.2	0.3	0.0	0.5	0.0	0.2	0.0
0.2 - 0.3	0.2	0.0	0.1	0.0	0.2	0.0
0.3 - 0.4	0.1	0.0	0.2	0.0	0.1	0.0
0.4 - 0.5	0.0	0.0	1.3	0.0	0.8	0.0
0.5 - 0.6	0.1	0.1	0.4	0.1	0.5	0.0
0.6 - 0.7	0.4	0.1	0.4	0.2	0.1	0.0
0.7 - 0.8	59.2	23.0	64.0	15.9	36.9	0.4
0.8 - 0.9	37.4	73.6	31.4	81.9	59.9	89.2
0.9 - 1.0	1.4	3.0	1.0	1.8	1.0	10.3

* Values given are the mean (n=3).

TABLE 6

DISTRIBUTION OF ^{14}C-ACTIVITY IN EXTRACTS OF FOLIAGE FROM SILVER MAPLE SAPLINGS TRUNK-INJECTED WITH ^{14}C-UNICONAZOLE

Fraction on Chromatography Plate Counted (Rf values)	Days After Injection			
	133		497	
	Extract	Uniconazole Reference	Extract	Uniconazole Reference
	%^{14}C-Activity detected on Plate*			
0.0 - 0.1	0.7	0.2	1.9	0.5
0.1 - 0.2	0.8	0.1	1.4	0.1
0.2 - 0.3	0.5	0.0	1.9	0.1
0.3 - 0.4	0.5	0.0	1.8	0.1
0.4 - 0.5	0.6	0.0	1.2	0.0
0.5 - 0.6	0.4	0.1	0.8	0.1
0.6 - 0.7	3.4	0.1	7.1	0.1
0.7 - 0.8	37.0	1.1	53.8	0.4
0.8 - 0.9	50.8	95.7	29.1	96.5
0.9 - 1.0	5.2	2.7	1.0	2.2

* Values given are the mean (n=3).

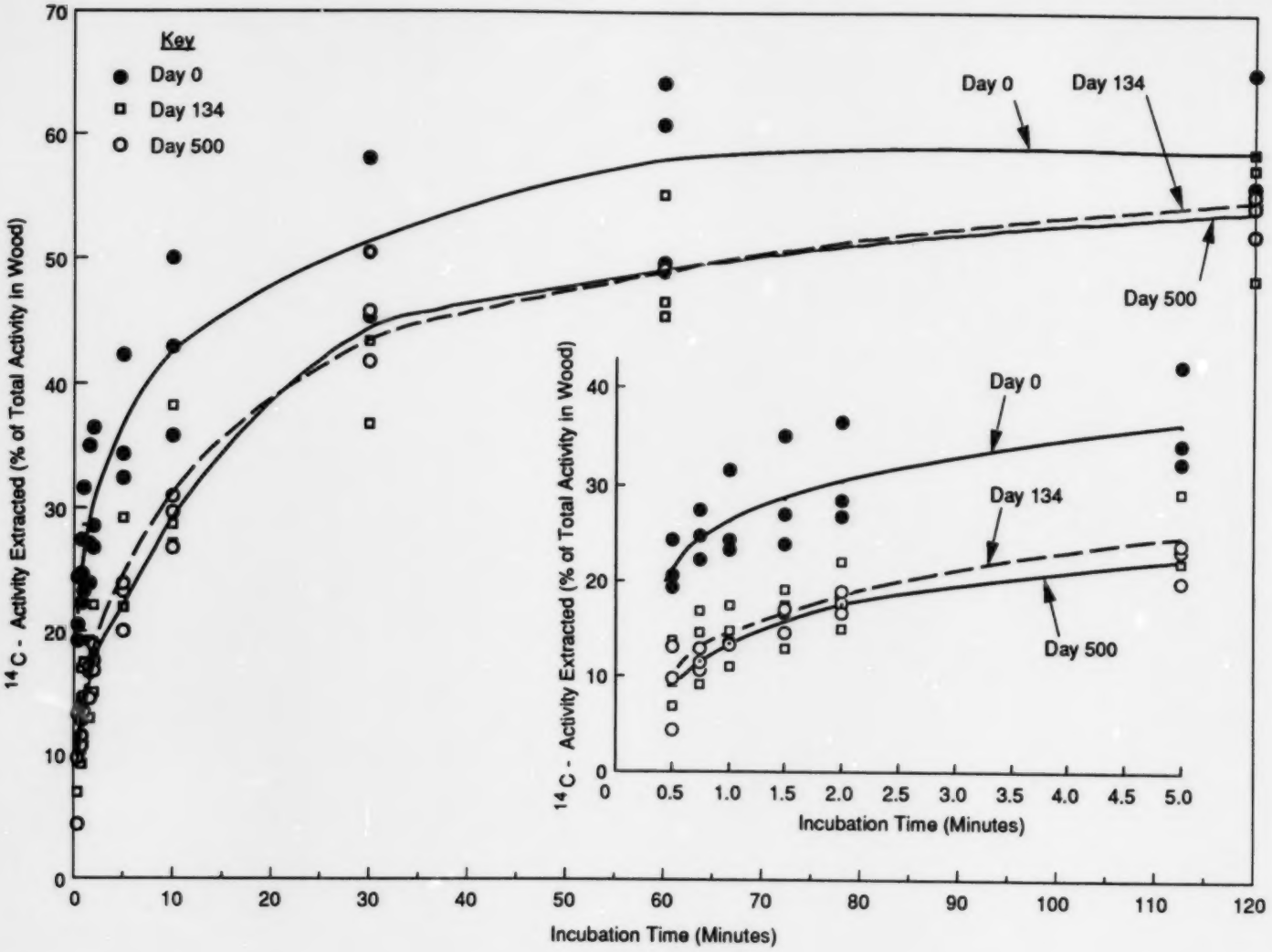

FIGURE 1

TIME - COURSE FOR THE EXTRACTION BY WATER OF ^{14}C - ACTIVITY FROM WOOD OF SILVER MAPLE SAPLINGS HARVESTED 0, 134 OR 500 DAYS AFTER TRUNK-INJECTION WITH ^{14}C - UNICONAZOLE
At each of the three harvest times, three saplings were harvested. Samples of wood from each sapling were incubated with water for 0.5 to 120 minutes and then the ^{14}C - activity in the water determined. Each point plotted on the graph represents one sapling, and the curves join the mean values calculated at each incubation time for three saplings in each harvest group.

incubation studies conducted here with a 'closed' system. Results from the metabolite determination experiments described previously showed that the ^{14}C-activity in the wood samples was associated with uniconazole and not metabolites, and thus in the time-course experiment it was uniconazole which was released.

In a second set of experiments a wide variety of solvents was used to extract samples of wood from the saplings harvested at the various times. All wood and solvent mixtures were incubated for 4 hours. The results from these extraction experiments are presented in Figure 2. The ^{14}C-activity extracted was expressed as a function of the dielectric constant (a measure of "solvent polarity") of the various solvents. With nonpolar solvents (low dielectric constants e.g., toluene, chloroform, ethyl acetate) there was a big difference between the ^{14}C-activity extracted from day 134 or 500 wood and day 0 wood, with between two and five times more ^{14}C-activity being extracted from the latter (see Figure 2). With more polar solvents (dielectric constants of 30 to 80) there was no apparent difference between the degree of extraction for the wood from saplings harvested at different times. The most efficient extractions were performed with 80% isopropanol and 80% methanol (dielectric constants of 30.4 and 41.8, respectively) with about 80% of the ^{14}C-activity being extracted. In the uniconazole/metabolite determination experiments described previously 80% methanol was used as the solvent in the extractions.

The ^{14}C-activity in the wood from day 0 saplings was more readily extracted by nonpolar solvents than the activity in wood from day 134 and 500 saplings. It would appear that during residence time in the sapling, changes occur in the accessability and/or binding of the growth regulator to wood components. An analysis of the extraction data for the various solvents also revealed that there appeared to be no direct relationship between the specific activity of the growth regulator in the wood (expressed as Bq/g dry weight) and the percentage of activity extracted by the solvents. These data are presented in Table 7. While the specific activity in the wood samples ranged from 139.7 to 565.9 Bq/g dry weight (top of table to bottom), there was no concomitant increase in the percentage of that activity that was extracted by water, 80% methanol or 80% isopropanol. Thus the ratio of accessible to nonaccessible (soluble vs. insoluble) uniconazole remains fixed, and is not dependent on the initial concentration of the uniconazole in the wood. This suggests that as the concentration of growth regulator declines in the wood with time (as a consequence of translocation) the same percentage of that growth regulator is always available. If this were to hold even at very low concentrations, it would constitute a more efficient system than one in which a significant portion of the growth regulator was unavailable immediately after application and remained unavailable with time. In the latter case, the translocation of uniconazole would result in the amount of available growth regulator diminishing (eventually to nothing). In both cases the amount of

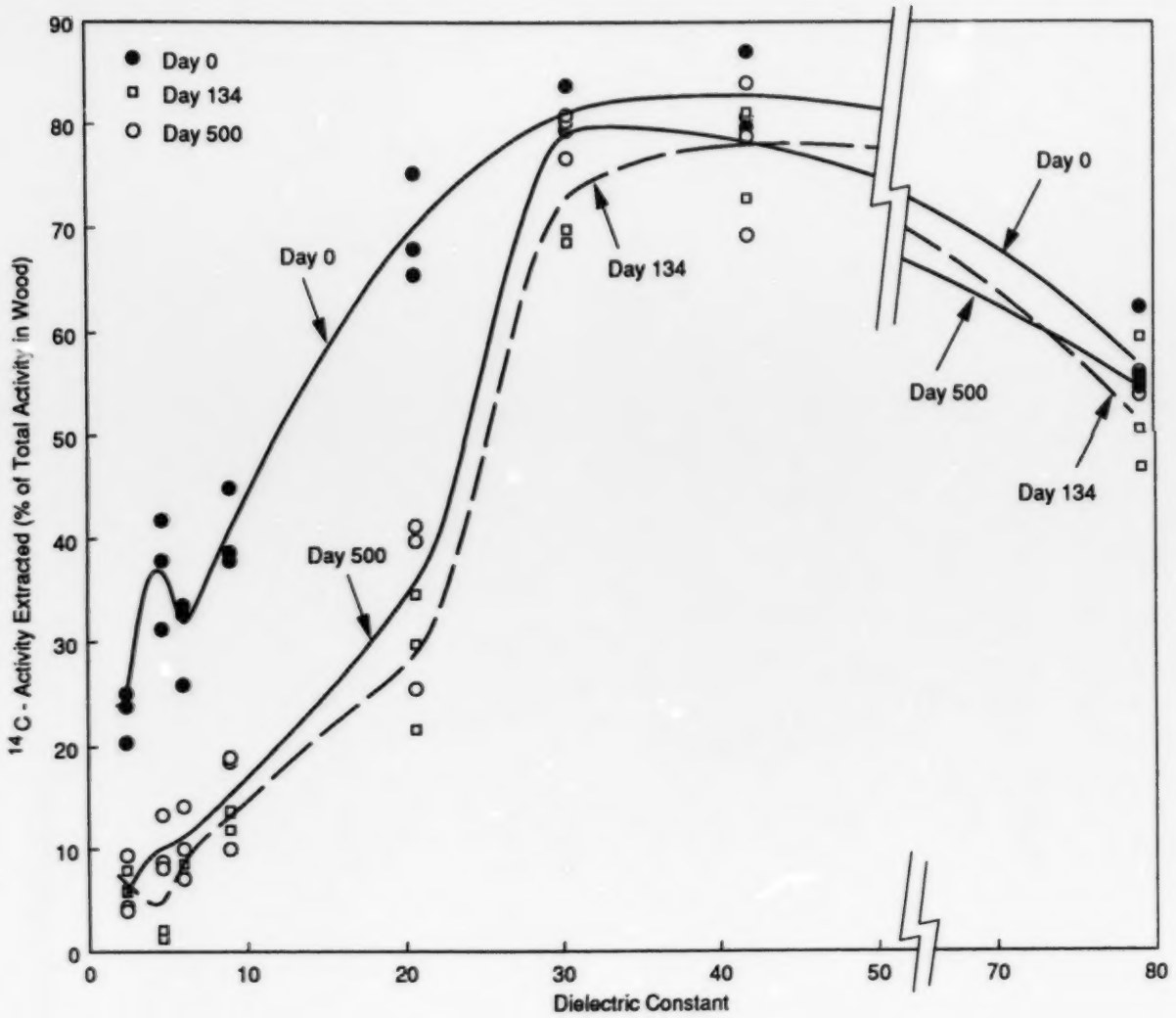

FIGURE 2

EXTRACTION BY VARIOUS SOLVENTS OF ^{14}C - ACTIVITY FROM WOOD OF SILVER MAPLE SAPLINGS HARVESTED 0, 134 OR 500 DAYS AFTER TRUNK-INJECTION WITH ^{14}C - UNICONAZOLE.
Each point plotted on the graph represents one sapling, and the curves join the mean values calculated for these saplings for each solvent. List of solvents provided in material and methods.

TABLE 7

RELATIONSHIP BETWEEN INITIAL ^{14}C-ACTIVITY IN WOOD AND THE PROPORTION OF THAT ACTIVITY EXTRACTED BY VARIOUS SOLVENTS

	DAYS AFTER INJECTION SAPLING HARVESTED											
	0	134	500	0	134	500	0	134	500	0	134	500
				SOLVENTS								
				WATER			80% METHANOL			80% ISOPROPANOL		
SAPLING	^{14}C-ACTIVITY (Bq/g dry weight)			%^{14}C-ACTIVITY EXTRACTED								
B3			139.7			56.1			84.1			80.9
F5		190.6			50.6			80.9			69.8	
C4		263.6			46.9			72.7			68.7	
B5			288.6			54.0			78.8			80.2
F4	371.9			55.2			79.5			79.5		
F1			418.9			55.2			69.2			76.7
C6		456.2			59.4			79.0			79.8	
E7	498.8			55.7			80.6			79.7		
B7	565.9			62.4			87.2			83.8		
Mean	478.9	303.5	282.4	57.8	52.3	55.1	82.5	77.6	77.4	81.0	72.8	79.3
(±s.d.)*	±98.5	±137.2	±139.7	±4.0	±6.4	±1.0	±4.2	±4.3	±7.5	±2.4	±6.1	±2.2

*Mean value calculated from three values in column above.
+Wood samples were extracted with either water, 80% methanol or 80% isopropanol as described in Materials and Methods. Data given for all three solvents are the same as in Figure 2.

growth regulator applied would be important to ensure that inhibition of growth was sustained for three or four years.

4.0 CONCLUSIONS

1. Following trunk injection of silver maple saplings with ^{14}C-uniconazole in May 1987, inhibition of sprout growth was observed in 1988 and 1989.

2. ^{14}C-activity detected in the foliage of the saplings increased in a linear manner in the 1987, 1988 and 1989 growing seasons. The maximum activity found in 1989 was about one sixth that attained in 1987 and one half that of 1988. As a function of the total ^{14}C-activity injected into the saplings, the foliage produced in 1989 contained 1.2 ± 0.2%, compared with 7.2 ± 1.5% and 2.3 ± 0.6% in 1987 and 1988 respectively.

3. In wood from just above the injection site of saplings harvested 0, 134 or 500 days after injection, between 95 and 97% of ^{14}C-activity detected was uniconazole. In foliage from saplings harvested at 133 or 497 days slightly less ^{14}C-activity chromatographed with uniconazole indicating little uniconazole metabolism.

4. In extraction experiments performed with water about 20% of the ^{14}C-activity present in wood from just above the injection site of saplings harvested immediately after injection was extracted by a 30 second incubation, while about 10% was extracted from wood of saplings harvested at 134 or 500 days. With longer incubation times this difference was not apparent.

5. Extraction experiments performed with other solvents also revealed that the time of harvest had an effect on the proportion of ^{14}C-activity which was extracted. With nonpolar solvents such as toluene, chloroform or ethyl acetate, a larger percentage of the initial ^{14}C-activity in the wood was extracted from wood of saplings harvested at day 0 than from wood of day 134 or 500 saplings.

6. The proportion of ^{14}C-activity that was extracted by the various solvents did not change as a function of the amount of ^{14}C-activity in the wood or the time post-injection the wood was harvested. This would suggest that as the concentration of uniconazole in the wood declines as a result of translocation, the same percentage of the growth regulator would still be available for translocation.

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